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Induction of Triploidy in Chinook Salmon (*Oncorhynchus tshawytscha*) using Hydrostatic Pressure

by

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and

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Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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ABSTRACT

Hydrostatic pressure shocking was applied to fertilized Chinook salmon (*Oncorhynchus tshawytscha*) eggs in 2003 and 2004 to induce triploidy. In 2003, eggs collected from Ship Creek Chinook salmon broodstock were pressure shocked for 4 minutes beginning at 240 or 400 Centigrade Temperature Minutes (CTMs) post fertilization with 9,000, 9,500, or 10,000 psi of pressure. The three treatments shocked at 400 CTMs each achieved an average eyed-egg survival rate ≥70% of the control group survival rate. None of the 240 CTM treatments achieved this goal. All treatment groups were tested for ploidy regardless of survival rates. Ten of the 12 individual treatment groups achieved 100% triploidy rates as measured by flow cytometry.

In 2004, treatment groups were shocked for 3 or 4 minutes with 10,000 psi of pressure beginning at 300 CTMs, 400 CTMs, or 500 CTMs post fertilization. Five of the six treatments achieved an average eyed-egg survival rate ≥70% of the control group survival rate. Of the 10 individual treatment groups tested for ploidy, 7 achieved a 100% triploidy rate.

Key words: triploid, flow cytometry, Chinook salmon, *Oncorhynchus tshawytscha*, survival, hydrostatic pressure, meiotic division, chromosomes.

INTRODUCTION

Alaska Department of Fish and Game (ADF&G) currently stocks diploid Chinook salmon (*Oncorhynchus tshawytscha*) in Southcentral and Interior Alaska lakes for ice fishing. These fish could establish a breeding population should they access a water body capable of anadromous fish production. Stocking sterile, triploid Chinook salmon would ensure that hatchery-released salmon removed from an ADF&G approved stocking location and illegally transported and stocked in a new location could not establish a breeding population. A secondary benefit to stocking triploid Chinook salmon may be preventing or delaying the onset of sexual maturity (Johnson et al. 1986). Diploid Chinook salmon support winter ice fisheries and are readily harvested, thus making the introduction of Chinook salmon to landlocked lakes a useful management tool to maintain fishing opportunity throughout the year. However, they tend to have a high proportion of precocious males at release. When these males mature, the associated secondary sexual characteristics are obvious, the body cavity is filled with milt to the point of deforming the fish, and they usually die.

Heat shock, cold shock, hydrostatic pressure, and nitrous oxide treatments are designed to produce sterile, triploid fish (Malison et al. 2001). These procedures prevent the occurrence of second meiotic division which results in two sets of chromosomes being contributed by the female and one set from the male. Successful triploidy depends largely on three factors: time of shock initiation (CTMs [Centigrade Temperature Minutes]), duration of the treatment, and the intensity of the treatment.

Westerhof (1988) achieved a 100% triploidy rate and 53.1% survival rate to hatching when Chinook salmon eggs were heat shocked and slowly cooled to ambient temperature. Teskeredzic et al. (1993) compared triploid yield rates (triploid rate x percent survival to hatch) of coho salmon (*O. kisutch*) eggs that were heat shocked to triploid yield rates for eggs that were pressure shocked and found lower triploid yield rates for heat-shocked eggs. Variation in egg size may result in undersized eggs receiving a greater than intended thermal shock and oversized eggs receiving an insufficient thermal shock. Because all eggs within an egg group experience the same pressure change when shocked, egg size does not affect triploidy rates of pressure-shocked eggs.

Hydrostatic pressure shocking has been used successfully to induce triploidy in various salmonid species including Arctic char (*Salvelinus alpinus*), coho salmon, and rainbow trout (*O. mykiss*)

(Table 1) (Eric Johnson, Icy Waters Ltd., personal communication, O'Keefe and Benfey 1995; Teskeredzic et al. 1993; Wickwire 2000).

Table 1.-Shock initiation time, pressure, and duration of shock used to achieve 100% triploidy rates in Arctic char, coho salmon, and rainbow trout.

Species	Centigrade Temperature Minutes	Pressure (psi)	Duration (min)	Reference
Arctic char	300	9,500	5	O'Keefe and Benfey 1995
coho salmon	210 420	10,000	4	Teskeredzic et al. 1993
rainbow trout	300	9,150 9,700 10,400	5	Wickwire 2000

Hydrostatic pressure was used to induce triploidy in Chinook salmon eggs at Fort Richardson Hatchery (FRH) in 2003 and 2004. The shock initiation times for the treatment groups in 2003 were based on successful shock initiation times used for coho salmon (Teskeredzic et al. 1993). Acceptable survival rates were not achieved by the egg groups shocked in 2003, so procedures were adjusted and a second series of shockings was completed in 2004.

The goal of this project was to create triploid Chinook salmon using hydrostatic pressure-shocking procedures. The specific objectives were to:

1. Estimate the mean survival rate from fertilization to the eyed-egg stage for each treatment group (Table 2).

For those treatment groups with a survival rate greater than or equal to 70% of the control group survival rate:

2. Determine which treatment produced the highest rate of triploidization.

METHODS

2003

Eggs and milt were collected from Ship Creek Chinook salmon broodstock on 21 July 2003 during the course of a production egg take. Approximately 45 ml of eggs collected from each of seven females were combined in a Ziploc® bag and stored in a cooler on ice. Milt collected from six males was stored in separate vials in a cooler on ice. The gametes were transported to FRH.

Milt was tested for sperm motility before fertilization by activating it with a 120 mM solution of NaCl. When sperm motility was verified in four milt vials, the remaining vials were excess and discarded without testing. Approximately half of the egg mixture was transferred to a plastic container for fertilization. These eggs were used for one replicate. Approximately an equal amount of milt from each of the four males was added, and sperm were activated with the saline

Table 2.-Treatment groups of Chinook salmon eggs used in the hydrostatic pressure experiment in 2003 and 2004.

	Treatment	Time from fertilization to shock (min)	Centigrade Temperature Minutes (CTMs)	Hydrostatic pressure (psi)	Pressure shocking duration (min)
2003					
	1	30	240	9,000	4
	2	30	240	9,500	4
	3	30	240	10,000	4
	4	50	400	9,000	4
	5	50	400	9,500	4
	6	50	400	10,000	4
	7	ND	ND	control group	
2004					
	1	37.5	300	10,000	3
	2	50.0	400	10,000	3
	3	62.5	500	10,000	3
	4	37.5	300	10,000	4
	5	50.0	400	10,000	4
	6	62.5	500	10,000	4
	7	ND	ND	control group	

Note: Time from fertilization to shock initiation is based on an 8.0°C water bath in 2003 and an 8.1°C water bath in 2004.

solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 8.0°C water 1 minute post fertilization.

The fertilized eggs were equally divided among seven egg tubes (each egg tube contained approximately 100 eggs): one for each of the six treatments and a control group. The tubes were submerged in an 8.0°C ±0.1°C water bath. At 8.0°C, the 240 CTM and 400 CTM egg groups water hardened for 30 and 50 minutes, respectively, before they were pressure shocked.

Eggs were pressure shocked in one of three stainless steel chambers fitted with a double O-ring brass piston. Each chamber was filled with 8.0°C water and the eggs from the 240 CTM treatment groups were added. The piston sealed the top of the chamber and air and excess water were expelled via a side relief valve before sealing the chamber. A 12-ton shop press with a 12-ton bottle jack was used to achieve 9,000 psi within one pressure chamber, a 15-ton shop press was used with a 20-ton jack to achieve 9,500 psi within a second pressure chamber, and a 25-ton press was used with a 25-ton jack to achieve 10,000 psi in a third pressure chamber. This procedure was repeated for the 400 CTM treatment groups.

The pressure shocking treatments began when the desired pressure was achieved within each chamber. After 4 minutes, each chamber was rapidly depressurized, and eggs were transferred back to the 8.0°C water bath. At 75 minutes post fertilization, the egg tube containing the control

group was placed in a non-pressurized chamber. The control eggs remained in the non-pressurized chamber for 4 minutes before being returned to the 8.0°C water bath. At 90 minutes post fertilization, all eggs were transferred from the egg tubes to individual incubation containers and disinfected with an iodophor solution (1:100 concentration) for 15 minutes.

During the 240 CTM shocking, a leaky needle valve in the 9,500 psi pressure chamber caused the pressure to decrease to 9,000 psi after 2 minutes and 20 seconds. The pressure was increased to 9,500 psi where it remained for the duration of the pressure treatment.

The process was repeated for the second replicate using milt from the same four males to fertilize the remaining eggs from the same mix of eggs used for replicate one. Each replicate of fertilized eggs was incubated in separate Heath trays.

Survival

At the eyed-egg stage, the eggs in each incubation container were physically shocked by pouring them into a container of water from a height of approximately 1 foot to rupture the vitelline membrane of dead or unfertilized eggs, causing them to turn white. Dead eggs were removed after 24 hours. Eggs were enumerated and classified as either alive or dead. The eggs in each incubation container were inspected again at hatching. Dead eggs were removed and attempts were made to separate and enumerate them as either eyed or blank. Live and dead fry and dead alevin were enumerated at emergence.

Simple binomial proportions were used to calculate the survival rate for each group, and treatment survival rates were estimated by averaging replicates.

Ploidy

Flow cytometry was used to analyze tissue cells for ploidy (Thorgaard et al. 1982). Muscle, skin, and fin tissue samples were collected from up to 45 fish in each treatment group and frozen in a 4', 6-Diamidino-2-phenylindole dihydrochloride hydrate (DAPI) solution with 10% Dimethyl sulfoxide (DMSO) (Thornthwaite et al. 1980). Each treatment group was tested for ploidy even though average eyed-egg survival rates were <70% of the control group survival rate for eggs shocked at 240 CTMs, and fewer than 45 individuals survived to emergence in 8 of the 12 treatment groups.

If the triploidization rate of the first 10 samples of a group was less than 80%, then no further samples from that group were tested.

The average triploidization rate for each treatment was calculated as a simple binomial proportion averaged across replicates. For those treatments with an average survival rate ≥70% of the control group, the average triploidization rates were ranked to determine the most effective treatment.

2004

Eggs and milt were collected from Ship Creek Chinook salmon broodstock on 22 July 2004 and transported to FRH as described for 2003. Sperm motility testing, transferring the egg mixtures for replicates one and two, and fertilization procedures were also conducted as described for 2003.

The fertilized eggs were equally divided among seven egg tubes: one for each treatment and a control group. The tubes were submerged in an 8.1°C ±0.1°C water bath. At 8.1°C, the 300

CTM, 400 CTM, and 500 CTM egg groups water hardened for 37, 49.4, or 61.7 minutes, respectively, before they were pressure shocked.

Eggs were pressure shocked in one of two stainless steel chambers fitted with a double O-ring brass piston. Each chamber was filled with 8.1°C water, and eggs from the 300 CTM treatment groups were added. The piston sealed the top of the chamber, and air and excess water were expelled via a side relief valve before sealing the chamber. A 15-ton shop press was used with a 20-ton jack to achieve 10,000 psi for 4 minutes in one pressure chamber, and a 25-ton jack was used with a 25-ton press to achieve 10,000 psi for 3 minutes in a second pressure chamber. This procedure was repeated for the 400 CTM and 500 CTM treatment groups.

The pressure shocking treatments began when the desired pressure was achieved within each chamber. Once the 3- or 4-minute shock was achieved, the chambers were depressurized and the eggs were transferred back to the 8.1°C water bath. At 75 minutes post fertilization, the egg tube containing the control group was placed in a non-pressurized chamber. The control eggs remained in the non-pressurized chamber for 4 minutes before being returned to the 8.1°C water bath. At 105 minutes post fertilization, all eggs were transferred to an iodophor solution (1:100 concentration) for a 15-minute disinfection. Disinfected eggs were then transferred to an incubation container within a Heath tray.

A leaky O-ring in the 25-ton jack caused the pressure in the 3-minute shock chamber to drop below 10,000 psi when unattended, but when continuous pressure was applied throughout the treatment the pressure in the chamber remained at or near 10,000 psi. A 12-ton press and hydraulic jack replaced the 25-ton press and jack for the 3-minute shock chamber used for the second replicate.

The fertilization and shocking processes were repeated for replicate two using milt from the same four males and the same mix of eggs used in replicate one. Each replicate of fertilized eggs was incubated in separate Heath trays.

Survival

At the eyed-egg stage, eggs in each incubation unit were physically shocked, dead eggs removed, and enumerated as described for the 2003 egg groups. The presence of fungus necessitated the removal of dead eggs at hatching. Attempts were made to separate and enumerate the dead eggs as either eyed or blank. Live and dead fry and dead alevin were enumerated at emergence.

Simple binomial proportions were used to calculate the survival rate for each group, and treatment survival rates were estimated by averaging across replicates.

Ploidy

Blood and slime cells collected from Chinook salmon treatment groups in 2004 were preserved and analyzed as described for the 2003 tissue samples.

Treatment groups were tested for ploidy if the average estimated survival rate to the eyed-egg stage for that treatment was at least 70% of the control group survival rate.

If the triploidization rate of the first 10 samples of a group was less than 80%, then no further samples from that group were tested. The average triploidization rate for each treatment was calculated as a simple binomial proportion averaged across replicates. For those treatments with an average survival rate $\geq$70% of the control group, the average triploidization rates were ranked to determine the most effective treatment.

RESULTS

2003

Survival

Survival rates to the eyed-egg stage for individual treatment groups were less than 35% for treatment groups shocked at 240 CTMs, and ranged from 68.8 to 82.6% for the groups shocked at 400 CTMs, and 96.2 to 98.5% for the control groups (Tables 3 and 4).

Ploidy

Embryos from each treatment group were tested for ploidy even though only the groups shocked at 400 CTMs achieved the minimum eyed-egg survival rate criteria, and only 4 of the 12 treatment groups had enough embryos survive to meet the minimum sample size requirements of 45 individuals. Triploidy rates ranged from 95.5 to 100% (Table 3).

2004

Survival

Survival rates to the eyed-egg stage for individual treatment groups ranged from 53.5 to 75.8% for treatment groups shocked at 300 CTMs, 88.4 to 93.1% for groups shocked at 400 CTMs, 91.6 to 98.4% for groups shocked at 500 CTMs, and 98.8 to 99.5% for the control groups (Tables 5 and 6).

Ploidy

Embryos from 10 of the 12 treatment groups were tested for ploidy. Triploidy rates ranged from 86.7 to 100% (Table 5).

DISCUSSION

Successfully inducing triploidy using hydrostatic pressure depends largely on three factors: time of shock initiation (CTMs), intensity of pressure used, and duration of the treatment. For each pressure treatment (9,000 psi, 9,500 psi, and 10,000 psi) the average survival rate to the eyed-egg stage of Chinook salmon eggs shocked at 240 CTMs was at least 58% lower than those shocked at 400 CTMs. Although two of the three treatments shocked at 240 CTMs achieved estimated triploidy rates of 100%, the mortality rate of approximately 75% for those groups suggests that most eggs were not at the proper developmental stage for successfully inducing triploidy or for surviving the shock.

Chinook salmon eggs from broodstocks incubated at FRH require more Centigrade Temperature Units to reach the eyed-egg stage and hatch than do the eggs of rainbow trout, Arctic char, coho salmon, or Arctic grayling (*Thymallus arcticus*) (Table 7). It is reasonable to assume that to induce triploidy and achieve acceptable survival rates, Chinook salmon eggs would require more CTMs between fertilization and pressure shocking than these other species.

The shock initiation times of 300 CTMs, 400 CTMs, and 500 CTMs tested in 2004 repeated and bracketed the 400 CTM times tested in 2003. The survival rates of eggs shocked for 3 minutes at 400 and 500 CTMs were at least 50% higher than those shocked at 300 CTMs. The survival rate of eggs shocked for 4 minutes was 27% higher at 400 CTMs and 35% higher at 500 CTMs than those shocked at 300 CTMs. However, only the 400 CTM groups consistently achieved 100% triploidy rates, whereas some 500 CTM replicates did not. At 500 CTMs post fertilization, some Chinook salmon eggs may have developed beyond the stage needed to induce triploidy. The

average estimated survival and triploidy rates of shocked eggs indicate that eggs are not developed enough at 300 CTMs post fertilization to successfully induce triploidy.

In 2003, the 10,000 psi treatment groups achieved a 100% tripoidy rate, and had the highest average estimated survival rates of the three treatments shocked at 400 CTMs. All treatment groups in 2004 were shocked with 10,000 psi of pressure.

Table 3.-Survival and triploidization rates for pressure-shocked treatment groups of Chinook salmon eggs in 2003.

	Treatment		Percent survival			
Replicate	CTMs	Pressure	Green to eyed egg	Green egg to emergence	Eyed egg to emergence	% triploids in sample
1	240	9,000	33.0%	27.5%	83.3%	100.0%
1	240	9,500	30.4%	27.5%	90.3%	100.0%
1	240	10,000	33.0%	25.5%	77.4%	100.0%
1	400	9,000	71.1%	44.6%	62.8%	100.0%
1	400	9,500	69.5%	53.4%	76.8%	100.0%
1	400	10,000	82.6%	66.1%	80.0%	100.0%
1	Control		98.5%	97.8%	99.2%	
2	240	9,000	25.0%	22.0%	88.0%	100.0%
2	240	9,500	23.6%	21.7%	92.0%	95.5%
2	240	10,000	21.1%	14.7%	70.0%	100.0%
2	400	9,000	68.8%	67.7%	98.4%	97.6%
2	400	9,500	75.0%	54.2%	72.2%	100.0%
2	400	10,000	69.8%	63.5%	91.0%	100.0%
2	Control		96.2%	90.4%	94.0%	

Table 4.-Average survival and triploidization rates for pressure-shocked treatments of Chinook salmon eggs in 2003.

		Eyed egg		Green egg to emergence		Eye to emergence		
CTMs	Pressure	Survival to	Relative to control	Survival to	Relative to control	Survival to	Relative to control	Percent triploid
240	9,000	29.0%	29.8%	24.8%	26.3%	85.7%	88.7%	100.0%
240	9,500	27.0%	27.7%	24.6%	26.1%	91.2%	94.3%	97.7%
240	10,000	27.0%	27.8%	20.1%	21.4%	73.7%	76.3%	100.0%
400	9,000	69.9%	71.9%	56.2%	59.7%	80.6%	83.4%	98.8%
400	9,500	72.2%	74.2%	53.8%	57.2%	74.5%	77.1%	100.0%
400	10,000	76.2%	78.3%	64.8%	68.9%	85.5%	88.5%	100.0%
Control		97.3%	100.0%	94.1%	100.0%	96.6%	100.0%	

Table 5.-Survival and triploidization rates for pressure-shocked treatment groups of Chinook salmon eggs in 2004.

	Treatment		Percent survival			
Replicate	CTMs	Duration	Green to eyed egg	Green egg to emergence	Eyed egg to emergence	% triploids in sample
1	300	3[a]	66.7%	50.5%	75.7%	NS[c]
1	300	4	75.8%	67.0%	88.4%	100.0%
1	400	3[a]	88.4%	79.4%	89.8%	100.0%
1	400	4	90.4%	86.5%	95.7%	100.0%
1	500	3[a]	91.6%	68.0%	74.2%	86.7%
1	500	4	98.4%	91.4%	92.9%	97.8%
1	Control		99.5%	85.9%	86.3%	
2	300	3	53.5%	39.0%	72.8%	NS[c]
2	300	4[b]	68.4%	63.1%	92.2%	95.6%
2	400	3	93.1%	85.1%	91.4%	100.0%
2	400	4	92.7%	89.3%	96.3%	100.0%
2	500	3	96.2%	89.7%	93.2%	100.0%
2	500	4	96.3%	88.8%	92.2%	100.0%
2	Control		98.8%	98.2%	99.4%	

[a] Hydraulic jack did not maintain the pressure at 10,000 psi because of an oil leak. Attempts were made to manually maintain the pressure at 10,000 psi.

[b] Accidentally sampled for ploidy even though eyed egg survival rate was < 70%.

[c] Not sampled: eyed egg survival rate < 70%.

Table 6.-Average survival and triploidization rates for pressure-shocked treatments of Chinook salmon eggs in 2004.

		Eyed egg		Green egg to emergence		Eye to emergence		
CTMs	Duration (min)	Survival to	Relative to control	Survival to	Relative to control	Survival to	Relative to control	Percent triploid
300	3	60.1%	60.6%	44.7%	48.6%	74.3%	80.0%	
300	4	72.1%	72.7%	65.1%	70.7%	90.3%	97.3%	97.8%
400	3	90.7%	91.5%	82.2%	89.3%	90.6%	97.5%	100.0%
400	4	91.6%	92.3%	87.9%	95.4%	96.0%	103.4%	100.0%
500	3	93.9%	94.7%	78.8%	85.6%	83.7%	90.1%	93.3%
500	4	97.3%	98.2%	90.1%	97.8%	92.5%	99.7%	98.9%
Control		99.2%	100.0%	92.1%	100.0%	92.9%	100.0%	

Table 7.-Approximate Centigrade Thermal Units (CTUs) required to achieve the eyed-egg stage and hatching for rainbow trout, Arctic char, coho salmon, Chinook salmon, and Arctic grayling for fish stocks currently reared at Fort Richardson and Elmendorf fish hatcheries.

Species	Number of CTUs to eyed-egg stage[a]	Number of CTUs to hatching[a]
rainbow trout	240	300
Arctic char	230	413
coho salmon	265	455
Chinook salmon	330	490
Arctic grayling	≈100	≈185-200

[a] CTU information from Fort Richardson and Elmendorf hatcheries incubation records; CTUs to eyed-egg stage or hatching may be different for different stocks of fish.

In 2004, the hydraulic jack used for the first replicate of the 3-minute treatment groups developed an oil leak that caused the pressure in the chamber to drop below 10,000 psi. Although additional pressure was manually applied throughout the treatment, the low (86.7%) triploidy rate for the first replicate 500 CTM treatment group may have been the result of unsteady pressure. The 100% triploidy rate for the first replicate 400 CTM 3-minute treatment group indicates the pressure for this group was sufficient despite the leaky jack. A different jack and press were used for the second replicate 3-minute treatment groups, and a 100% triploidy rate was achieved by the second replicate 400 and 500 CTM treatment groups. The 300 CTM 3-minute treatment groups were not tested for triploidy because their survival rate was <70% of the control group.

Hydrostatic pressure shock treatments of 3 and 4 minutes appear to be sufficient to induce 100% triploidy rates, but average survival rates from green egg to emergence for groups shocked for 4 minutes were 6.9-45.5% higher than the 3-minute shock groups. In 2004, the 3-minute and 4-minute treatments shocked at 400 CTMs were the only treatments to achieve 100% triploidy rates. The 4-minute treatment groups had average estimated survival rates to the eyed-egg stage 0.9% higher and emergence rates 6.9% higher than the 3-minute treatment groups.

In 2003, only the three 400 CTM treatments achieved the minimum criteria for acceptable average survival rates (≥70% of the control group). Thus, only those treatments were included in the ploidy portion of this study, although all treatments were tested. In 2004, all treatments except the 300 CTM 3-minute treatments achieved the minimum survival criteria. In 2003 and 2004, only the 400 CTM treatments achieved 100% triploidy rates and had acceptable survival rates. The 10,000 psi treatment group shocked for 4 minutes beginning at 400 CTMs post fertilization had the highest survival rate in both years.

The average estimated survival rate from green egg to emergence for the 400 CTM 4-minute treatment at 10,000 psi was only 68.9% in 2003, and 95.4% in 2004. However, the average estimated survival rate from green egg to emergence for the diploid control groups was 94.1% in 2003 and 92.1% in 2004. In 2003, eggs were disinfected 15 minutes earlier and transferred to incubation containers 30 minutes earlier than eggs in 2004. Handling the eggs earlier in the water-hardening process may have negatively affected triploid survival rates in 2003.

CONCLUSIONS

1. Exposure to the disinfectant and/or the handling of triploid eggs at 90 minutes post fertilization may have contributed to egg mortality in the 2003 treatment groups. Triploid Chinook eggs should water harden in incubation water for 105 minutes before being disinfected, and should remain in the egg tubes for 120 minutes post fertilization.

2. Chinook salmon eggs pressure shocked for 4 minutes at 10,000 psi beginning at 400 CTMs post fertilization had the highest survival rate of all treatments that achieved a 100% triploidy rate. This treatment will be used to induce triploidy in future production lots of Chinook salmon.

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